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Title: Establishment of an Ex Vivo Lung Perfusion Rat Model for Translational Insights in Lung Transplantation

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes.**

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

   Our microscope is equipped with a camera port. Microscope Leica M651 (Camera Hitachi HV-D30).

   SCOPE: 2.6.2 and 2.7.
   Videographer: Please film all shots labeled SCOPE using a SCOPE kit

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes.**

   Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: https://review.jove.com/account/file-uploader?src=20124253

3. **Filming location:** Will the filming need to take place in multiple locations? **No.**

Current Protocol Length

Number of Steps: 15
Number of Shots: 40

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Paolo Oliveira:** This study establishes a reproducible rat EVLP model to investigate ischemia-reperfusion injury, inflammatory signaling, and donor graft function, aiming to evaluate, preserve, and repair lungs ex vivo for improved transplantation outcomes.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What are the most recent developments in your field of research?

1.2. **Paolo Oliveira:** The most recent developments include using EVLP to assess, recondition, and even treat donor lungs before transplantation.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.2*

What research gap are you addressing with your protocol?

1.3. **Paolo Oliveira:** Our protocol addresses the need for cost-effective, reproducible models for translational lung transplant research.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.13*

How will your findings advance research in your field?

1.4. **Paolo Oliveira:** Our findings provide a reliable small-animal EVLP model to test new therapies and improve donor lung evaluation and preservation.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.2*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Care Committee at the University Health Network

Protocol

2. Ex Vivo Lung Perfusion System of Rat Lungs

Demonstrator: Paolo Oliveira

2.1. To begin, check that all transducers are connected to the isolated perfusion organ equipment and the data acquisition system **[1]**. Then launch the data acquisition software **[2].**

2.1.1. WIDE: Talent checking the connection of transducers to the isolated perfusion organ equipment and data acquisition system

2.1.2. **SCREEN :** The data acquisition software is being launched. **NOTE**: Add missing media title card

2.2. Prior to perfusion, fill the *ex vivo* lung perfusion or EVLP *(E-V-L-P)* circuit with 150 milliliters of supplemented Steen solution **[1-TXT].** Set the warm water bath to 20 degrees Celsius **[2]** and circulate the water to warm up the EVLP system **[3].**

2.2.1. Talent fills the EVLP circuit with the supplemented Steen solution. **TXT: Steen Solution: Sodium Heparin (1000 USP); Cefazolin (50 mg); Methylprednisolone (50 mg)**

2.2.2. Talent sets the water bath to 20 °C.

2.2.3. Shot of water being circulated into the EVLP system.

2.3. Orotracheally intubate an anesthetized rat **[1-TXT]**. Next, connect the tracheal tube to the small-animal ventilation system to ventilate the rat **[2]**.

2.3.1. Talent intubates an anesthetized rat orotracheally. **TXT: Anesthesia: Ketamine (50 mg/kg) – Xylazine (5 mg/kg) Injection (i.p)**

2.3.2. Shot of the tracheal tube being connected to the small animal ventilation system. VIDEOGRAPHER'S NOTE: 2.3.2 – take 2 shows monitor
AND
TEXT ON PLAIN BACKGROUND:
Tidal Volume: 10 mL/kg
Rate: 60 breaths/min
FiO_2: 0.5
PEEP: 2 cm H_2O
Video Editor: Please place both shots side by side

2.4. Place the rat in a supine position **[1]**. With a pair of scissors and forceps, enter the peritoneal cavity **[2]** and carry the incision cranially to perform a median laparotomy **[3].**

2.4.1. Talent places the rat in a supine posture.

2.4.2. Shot of the peritoneal cavity being entered into. VIDEOGRAPHER'S NOTE: 2.4.2 – includes all shots until 2.5.1

2.4.3. Shot of the incision being increased cranially to perform a median laparotomy.

2.5. Now, inject sodium heparin into the portal vein **[1].** Enter the thoracic cavity through the xyphoid process **[2].** and cautiously open the diaphragm radially, without damaging the lung **[3].** Then cranially resection the sternum **[4].**

2.5.1. Talent injects sodium heparin into the portal vein.

2.5.2. The thoracic cavity is being entered via the xyphoid process. VIDEOGRAPHER'S NOTE: 2.5.2 – slated as 2.5.3, include shots until 2.5.4

2.5.3. The diaphragm is being opened radially.

2.5.4. The sternum is being resectioned.

2.6. To retrieve the heart and lung block, make an incision on the inferior vena cava, along the apex of the left heart **[1].** With a pair of micro-scissors, make an anterior incision in the right ventricular outflow tract **[2].**

2.6.1. Shot of an incision being made on the IVC, along the apex of the left heart. VIDEOGRAPHER'S NOTE: 2.6.1 – changed to scope shot, includes all scope shots until 2.7.2, look for shot in scope folder

2.6.2. SCOPE: Talent makes an incision on the right ventricular outflow tract with microscissors.
Videographer: Please film this shot using a SCOPE kit

2.7. Then insert an 18-gauge intravenous catheter into the pulmonary trunk [**1]** and flush the lungs with 20 milliliters of low potassium dextran or LPD *(L-P-D)* solution **[2-TXT].**

2.7.1. SCOPE: Talent inserts an 18 G intravenous catheter into the pulmonary trunk.

2.7.2. SCOPE: Shot of LPD being injected into the lungs. **TXT: LPD solution with prostaglandin E1 (10 mg/mL)**
Videographer: Please film these shots using a SCOPE kit

2.8. Immediately after flushing, clamp the lower third of the trachea at the end of inspiration to preserve the lungs in an inflated state **[1].** Then harvest the lung **[2]** and place it in LPD solution for storage **[3].**

2.8.1. Shot of the trachea being clamped for lung preservation. VIDEOGRAPHER'S NOTE: 2.8.1 – combined with 2.8.2

2.8.2. Shot of the lung being harvested.

2.8.3. Shot of the lung being placed in LPD solution.

2.9. To begin lung perfusion, first secure a pre-tied 0 *(zero)* silk ligature underneath the main pulmonary artery and around the ventricles **[1].** Connect the pulmonary artery cannula to the inflow line of the EVLP system **[2].**
2.9.1. Talent places a pre-tied 0 silk ligature under the main PA and ventricles.
2.9.2. Shot of the PA cannula being connected to the inflow line of the EVLP system. VIDEOGRAPHER'S NOTE: 2.9.2 – combined with 2.10.1

2.10. Start the peristaltic pump at 10% of maintenance flow, ensuring the removal of any air in the cannula **[1]**. Secure the inflow cannula into the main pulmonary artery **[2].** Then insert the drainage cannula from the apex of the heart into the left atrium **[3].**
2.10.1. Talent turns on the peristaltic pump to eliminate air in the cannula.
2.10.2. Talent places the inflow cannula into the main PA.
2.10.3. Shot of the drainage cannula being inserted from the heart apex into the left atrium. VIDEOGRAPHER'S NOTE: 2.10.3 – shot in 4K, includes all shots until 2.12.3, 2.11.2 and 2.12.1 shot order switched

2.11. With a pair of forceps, gently dilate the mitral valve, to aid the cannulation **[1]**. Connect the left atrial cannula to the outflow line of the system **[2].**
2.11.1. Shot of the mitral valve being gently dilated with a pair of forceps.
2.11.2. Talent connects the LA cannula to the outflow line of the system.

2.12. Now make a small hole in the trachea **[1]** and insert the tracheal cannula [**2].** Connect the tracheal cannula to the ventilation line of the system **[3]**.
2.12.1. Shot of a small hole being made in the trachea.
2.12.2. Talent inserts the tracheal cannula into the tracheal hole.
2.12.3. Shot of the tracheal cannula being connected to the ventilation line of the EVLP system.

2.13. Gradually increase the perfusion flow rate to attain 20% of the cardiac output **[1].** Release the tracheal clamp 20 minutes after perfusion initiation **[2].** Then start lung ventilation followed by EVLP gas flow **[3-TXT].**
2.13.1. **SCREEN:** The perfusion flow rate is being increased to 20% CO VIDEOGRAPHER'S NOTE: 2.13.1B – added shot in 4K, take 1 shows closing chamber, take 2 shows lung in chamber
2.13.2. Shot of the tracheal clamp being released. VIDEOGRAPHER'S NOTE: 2.13.2 – shot in 4K
2.13.3. **SCREEN:** The lung ventilation and EVLP gas flow is being initiated. **TXT: Maintain inflow perfusate PCO_2 between 35 - 45 mmHg NOTE**: Add missing media title card

2.14. Record the dynamic lung compliance and pulmonary vascular resistance every hour during EVLP **[1].** Take perfusate samples from the sample port every hour **[2].** Flash-freeze the samples in liquid nitrogen for further analysis **[3].**

2.14.1. **SCREEN:** The hourly dynamic lung compliance and pulmonary vascular resistance values are being seen, in a spreadsheet. **NOTE**: Add missing media title card

2.14.2. Talent extracts the perfusate samples from the sample port, using syringes.

2.14.3. Shot of the perfusate samples being placed in liquid nitrogen to flash freeze. VIDEOGRAPHER'S NOTE: 2.14.3 – take 1 and 2 transferring samples, take 3 placing in nitrogen

2.15. After ceasing perfusion, clamp the trachea to maintain the lungs in an inflated state **[1].** Then isolate lung samples in tubes **[2]**. Flash freeze the samples in liquid nitrogen **[3].**

2.15.1. Shot of the trachea being clamped to keep lungs inflated.

2.15.2. Talent isolates the lung samples into cryotubes.

2.15.3. Shot of the lung samples being placed in liquid nitrogen.

Results

3. Results

3.1. All lungs with CIT *(C-I-T)* ranging from 20 minutes to 18 hours could be perfused for 4 hours **[1-TXT].** Compliance gradually decreased in the 18-hour CIT group over the perfusion period **[2].**

3.1.1. LAB MEDIA: Figure 2 **TXT: CIT: Cold Ischemic Time**

3.1.2. LAB MEDIA: Figure 2A

3.2. No significant differences in vascular resistance, lung graft oxygenation, and glucose levels were observed for the groups **[1].** Lactate values tended to increase over time with higher values observed at longer CIT **[2].**

3.2.1. LAB MEDIA: Figure 2B-D *Video Editor: Please sequentially highlight images from B to D*

3.2.2. LAB MEDIA: Figure 2E *Video Editor: Please emphasize the black and dark blue curves*

3.3. All groups showed similar values of perfusion electrolytes **[1]** and level of edema formation, with the 24-hour group showing severe edema **[2].**

3.3.1. LAB MEDIA: Figure 2F-H

3.3.2. LAB MEDIA: Figure 2J *Video Editor: Please emphasize the image labeled 24-h CIT*

1. Transducer

Pronunciation link:
https://www.merriam-webster.com/dictionary/transducer
IPA (American): /trænˈdjuːsər/
Phonetic Spelling: tran-DYOO-sir

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2. Perfusion

Pronunciation link:
https://www.merriam-webster.com/dictionary/perfusion

IPA (American): /pərˈfjuː ʒən/
Phonetic Spelling: per-FYOO-zhuhn

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3. Acquisition (as in "data acquisition")

Pronunciation link:
https://www.merriam-webster.com/dictionary/acquisition
IPA (American): /ˌækwəˈzɪʃən/
Phonetic Spelling: ak-wuh-ZISH-uhn

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4. Ex vivo

Pronunciation link:
https://www.howtopronounce.com/ex-vivo
IPA (American): /ˌɛks ˈvaɪ.oʊ/
Phonetic Spelling: eks-VY-oh

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5. EVLP (abbreviation for Ex Vivo Lung Perfusion)

Pronunciation link:
No confirmed link found.
IPA (American): /i-i-vi-ɛl-pi/
Phonetic Spelling: ee-vee-el-pee

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6. Steen (as in Steen solution)

Pronunciation link:
https://www.xvivogroup.com/products-services/steen-solution/
(company page with proper name usage)
jhltonline.orgNICE+1lungbioengineering+6ScienceDirect+6NICE+6Biology Insights+5XVIVO+5xvivoperfusion.mkdev.nu+5
IPA (American): /stiːn/
Phonetic Spelling: steen

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7. Orotracheally

Pronunciation link:
No confirmed link found.
IPA (American): /ˌɔː.roʊ-trəˈkiː.əl.i/
Phonetic Spelling: OR-oh-truh-KEE-uh-lee

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8. Intubate

Pronunciation link:
https://www.merriam-webster.com/dictionary/intubate
IPA (American): /ɪnˈtuː.beɪt/
Phonetic Spelling: in-TOO-bayt

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9. Laparotomy

Pronunciation link:
https://www.youtube.com/watch?v=nywApm14uzw Cambridge Dictionary+4XVIVO+4SpringerLink+4YouTube
IPA (American): /ˌlæp.əˈrɒt.ə.mi/
Phonetic Spelling: lap-uh-ROT-uh-mee

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10. Xyphoid (as in xiphoid/xyphoid process)

Pronunciation link:
No confirmed link found.
IPA (American): /ˈzɪf.ɔɪd/
Phonetic Spelling: ZIF-oyd

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11. Heparin

Pronunciation link:
https://dictionary.cambridge.org/us/pronunciation/english/heparin
IPA (American): /ˈhɛp.ə.rɪn/
Phonetic Spelling: HEP-uh-rin en.wikipedia.org+6Cambridge Dictionary+6YouTube+6

12. Apex (as in “apex of the heart”)

Pronunciation link:
https://www.merriam-webster.com/dictionary/apex
IPA (American): /ˈeɪ.peks/
Phonetic Spelling: AY-peks

13. Cannula

Pronunciation link:
https://www.merriam-webster.com/dictionary/cannula
IPA (American): /ˈkæn.jə.lə/
Phonetic Spelling: KAN-yuh-luh

14. Ventilate (as in ventilation)

Pronunciation link:
https://www.merriam-webster.com/dictionary/ventilate
IPA (American): /ˈvɛn.tə.leɪt/
Phonetic Spelling: VEN-tuh-layt

15. Prostaglandin (as in “prostaglandin E_1”)

Pronunciation link:
https://www.merriam-webster.com/dictionary/prostaglandin
IPA (American): /ˌproʊs.təˈglæn.dɪn/
Phonetic Spelling: pros-tuh-GLAN-din